OBSERVATIONS ON NUCLEAR MIGRATION

AND HETEROKARYOTIZATION IN *ARMILLARIA*

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SUMMARY. — Heterokaryotization in compatible matings of *Armillaria* «species B» was studied with phase contrast microscopy and genetical methods. The velocity of nuclear migration is slow in comparison to the results obtained for other fungi. After the migrating nucleus has traversed a hypha, the cytoplasm and old nuclei largely disappear. Later on multinucleate cells appear. They are divided into binucleate cells from which the dikaryotic hyphae start by branching. After some irregular divisions, dikaryotic cells with clamp connections appear, but the nuclei in the tip cells soon fuse and the final result is hyphae with uninucleate diploid cells.

RESUMÉ. — Étude, à l'aide de la microscopie à contraste de phase et de méthodes génétiques, de l'hétérocaryotisation chez *Armillaria* «espèce B». La vitesse de migration des noyaux s'est révélée lente, par comparaison avec les résultats obtenus chez d'autres champignons. Après le déplacement du noyau en migration tout au long d'une hyphe, le cytoplasme et les noyaux âgés y disparaissent en grande partie. Plus tard, des articles multinucléés se forment: ils se cloisonnent en articles binucléés dont procèdent par ramification les hyphes dicaryotiques. Après quelques divisions irrégulières, des articles dicaryotiques bouclés apparaissent, mais les noyaux des articles apicaux fusionnent bientôt et l'on observe finalement des hyphes à articles diploïdes uninucléés.

The haploid phase in the life cycle of Hymenomycetes is represented by the basidiospore and the homokaryotic mycelium which grows from it. When two compatible homokaryotic mycelia meet each other, one or more cells fuse and the process of heterokaryotization starts. There are species in which the heterokaryotic hyphae arise almost directly from the fused cells (HARNACK, 1931), but in most of the species studied the process is more complicated.

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A phenomenon called nuclear migration forms an essential part of heterokaryotization (BULLER, 1931). The foreign nuclei invade the mycelium, divide repeatedly, associate with the original nuclei to form heterokaryotic cells and eventually the whole homokaryotic mycelium becomes heterokaryotic.

A prerequisite for nuclear migration in Hymenomycetes is partial disintegration of the hyphal septa. In *Schizophyllum* it is promoted by an enzyme, R-glucanase, which shows increased activity only in those mating combinations where different B incompatibility factors are involved (WESSELS and NIEDER-PRUEM, 1967). However, a comparable increase in the activity of this enzyme has not been detected in *Coprinus* (HAYLOCK et al., 1980).

Most of the evidence for nuclear migration has been obtained indirectly, using genetical methods to show the distribution of foreign nuclei in the mycelium (SNIDER, 1965; ROSS, 1976). Furthermore, direct observations have been made on migrating nuclei either in fixed preparations or in living hyphae with the aid of phase contrast microscopy (NIEDERPRUEM, 1980 a).

Recent studies on the *Armillaria mellea* complex have revealed an unusual life cycle with diploid nuclei in the vegetative mycelium (ANDERSON and ULLRICH, 1982). There is usually, however, a distinct but transient dikaryotic stage in the mating (KORHONEN and HINTIKKA, 1974; KORHONEN, 1978). In connection with the studies cited above, some observations were made on the migration of nuclei and heterokaryotization in *Armillaria*. These observations are presented here.

MATERIAL AND METHODS

Single-spore cultures, isolated from one fruit body of *Armillaria* «species B» (KORHONEN, 1978), were cultured at room temperature (20-22°C) on agar medium containing 1 or 0,1 % malt extract. For phase contrast microscopy, a thin layer of 0,1 % malt extract agar in a Petri dish was inoculated with two compatible single-spore isolates, at a distance of a few mm from each other. After the two cultures had come into contact the mycelia were covered with a cover glass and studied with ■ Wild M20 phase contrast microscope. For photomicrography, a Wild MEL 13 automatic microscope camera was used.

The rate of advancement of compatible nuclei in a haploid mycelium was estimated using genetical methods. A Petri dish was inoculated with a single-spore culture and covered with cellophane to prevent the growth of aerial mycelium. After 4 weeks, when the culture had reached a diameter of 40-50 mm, the cellophane was removed and the culture inoculated with a compatible isolate in the middle or at the margin of the colony. Small pieces of the mycelium were removed at different distances from the point of inoculation after 10 and 33 days. They were transferred to a new medium, cultured for 3 weeks and the diploidy or haploidy of the mycelium concluded from the external appearance of the colony (KORHONEN and HINTIKKA, 1974).
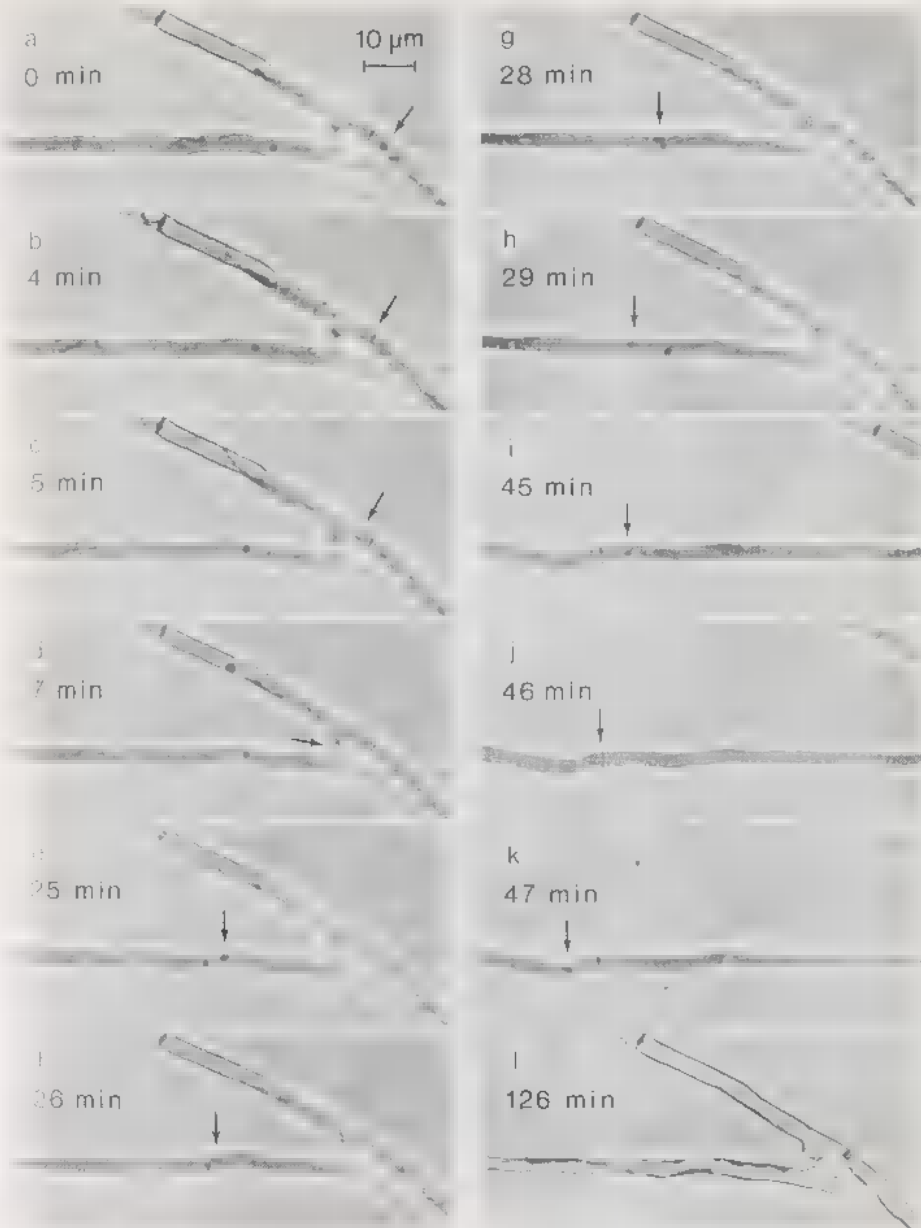
Fig. 1. – A compatible nucleus migrates proximally in a homokaryotic mycelium. It traverses a partially dissolved septum (a-c), passes along a fusion bridge to another hypha of the same mycelium (d), passes a local nucleus (e-h), and moves through the next septum (i-k). At a later stage (1) the migration hyphae are almost filled with vacuoles but not dead. Time elapsed from the first picture is indicated. The arrow indicates the nucleolus of the migrating nucleus. The exposure time is 0.5-1 sec.

RESULTS

After the advancing fronts of the compatible mycelia had met each other, the first cell fusions usually developed about one day later. The details of the fusion were not seen. In one recorded case nuclear migration started about 3 hours after cell fusion.

As the migrating nucleus proceeded through the hyphae, partial disintegration of the septa took place in front of it. Only those septa situated on the migration route were dissolved while those situated in branches outside the migration route, although very close to it, appeared to remain intact (Fig. 1).

The migrating nucleus traversed the disintegrated septa (Fig. 1, a-c and i-k), passed by local nuclei (Fig. 1, e-g) and divided now and then, but not in every cell. According to a few recordings, division of the migrating nucleus took place about every two hours whereas the division of nuclei in actively growing tip cells under similar conditions takes place once about every 5 hours. A complete septum was formed at the point where the migrating nucleus divided, often close to an old disintegrated septum.

Rather fundamental reorganization seemed to take place in the migration hyphae after the migrating nucleus had passed them. The old homokaryotic cytoplasm largely disappeared and the hyphae became heavily vacuolated (Fig 1, l). At a later stage multinucleate cells could be found in the migration hyphae (Fig. 2). They divided in some unknown way into short cells containing two compatible nuclei. These cells started to grow as branches of the main hypha and together formed a dense mycelial aggregate. The first divisions in the branches were more or less irregular. Often they were so called 1-2-1 divisions (KORHONEN and HINTIKKA, 1974). The normal conjugate divisions with clamp formation started later on, and, even later, the somatic diploidization took place in the dikaryotic tip cells (KORHONEN and HINTIKKA, 1974).

The general rate of advancement of diploidization in a haploid mycelium as estimated by genetical methods, varied between 0,7 and 2,0 mm/day (30 80μm/h) with an average of 1,1 mm/day (45μm/h; 25 determinations). The migration proceeded at about an equal rate in the proximal and distal direction in the mycelium. The momentary velocity of a migrating nucleus, as estimated from Fig. 1, a-k, is approximately 170μm/h, or about 4 mm/day. It should be noted that the nucleus did not divide during this movement. The growth rate of haploid and diploid hyphal tips under similar conditions was 0,5 0,8 mm/day, depending on the strain.

DISCUSSION

The observations presented above on the heterokaryotization of *Armillaria* are fragmentary only and many details remain unsolved. In general, the hetero

karyotization process in *Armillaria* resembles that of *Clitocybe truncicola* (BISTIS, 1970), as is to be expected on the basis of the relatedness of these two mushrooms.

Besides compatible matings, disintegrated septa can also be found in one hemicompatible mating factor combination of *Armillaria*. In this case, the hyphae with incomplete septa are usually restricted to a narrow zone between the paired mycelia (KORHONEN, 1978).

SNIDER (1965) collected the data available on the rate of nuclear migration in fungi. The recorded values for the Basidiomycetes *Coprinus*, *Schizophyllum*,
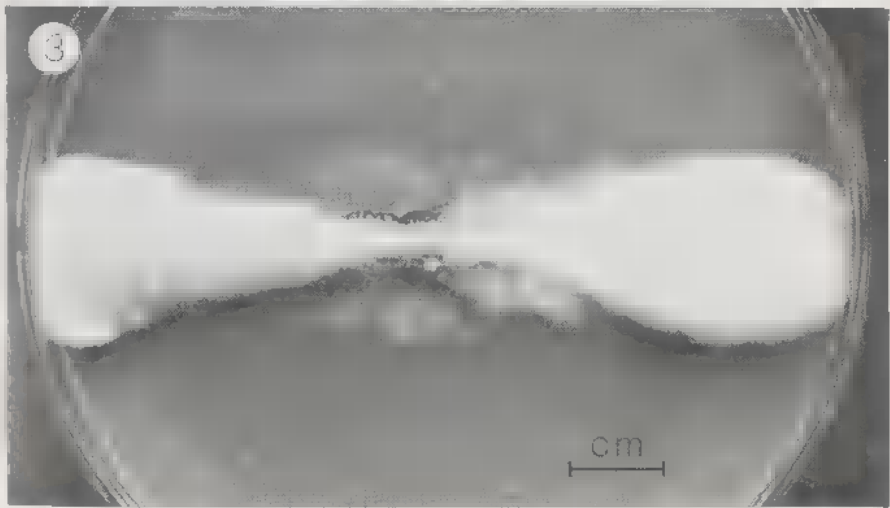

Fig. 2. — A multinucleate cell in a migration hypha. Stained with HCl-Giemsa (KORHONEN and HINTIKKA, 1974).

Fig. 3. — The spread of diploid mycelium into its haploid component mycelia. The Petri dish has been inoculated with two compatible single-spore isolates, both of them as a long thin inoculum from the middle to the margin of the dish. The diploid mycelium, which does not produce many aerial hyphae, spreads slowly from the middle towards the margins. Spreading is, however, 2-3 times faster than the growth of the hyphae. Age of culture 25 days.

and *Cyathus* are in the range of 0.37 to 3.2 mm/h. Later, ROSS (1976) reported a very high migration rate in *Coprinus congregatus* : 40 mm/h or more in young (3-7 days old) mycelia. The rate was very much dependent on the age of the mycelium, and in a 14 days old mycelium, the migration rate fell to 1.25 mm/h.

Compared with these data, the recorded rate of nuclear migration and heterokaryotization in *Armillaria* is low. It may be somewhat faster in inter-stock matings (KORHONEN, 1978) which were not investigated in this study. Although slow, the rate of heterokaryotization is usually 2-3 times faster than the growth rate of hyphal tips (Fig. 3). Only the growth rate of rhizomorphs may exceed the rate of nuclear migration, but haploid cultures of *Armillaria* produce few rhizomorphs, as compared with diploid ones.

The nuclear migration observed in *Armillaria* was not associated with streaming or pulsation of the cytoplasm, or the movement of other organelles visible under the phase contrast microscope. It is generally assumed that cytoplasmic microtubules play a role in nuclear movements but the exact mechanism is so far unclear (NIEDERPRUEM, 1980 a).

An interesting and largely unsolved question is what happens in the migration hyphae after the migrating nucleus has passed them. Typical phenomena for this stage are heavy vacuolization, as well as irregularities in the septation and in the number of nuclei per cell (BISTIS, 1970; RAUDASKOSKI, 1973; NIEDERPRUEM, 1980 b). BISTIS (1970) suggested that the change from stable homokaryon to stable dikaryon may involve the gradual synthesis of a new form of cytoplasm. The analysis of proteins support this interpretation : the proteins of a dikaryon are quite different to those of component homokaryons, even in the case where the homokaryons are very isogenic (ROSS et al., 1973).

In many of the species studied, the first divisions of dikaryotic cells formed from the migration hyphae show irregularities, and the normal conjugate divisions take place only after some time has passed (BISTIS, 1970; RAUDASKOSKI, 1973). At this stage the coordination between the two nuclei, or between the nuclei and the cytoplasm, is apparently incomplete. A comparable lack of coordination can be seen in a uninucleate hyphal cell isolated from a dikaryotic mycelium (KORHONEN and HINTIKKA, 1974). It is interesting to note, on the other hand that there are species in which the dedikaryotization of hyphal cells, without conidial formation, apparently takes place easily (ARITA, 1979).

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